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III. HYPOXYLON CANKER OF POPLAR

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II. The biology of *Fomes pinicola* (Sw.) Cooke by Irene Mounce.

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STUDIES IN FOREST PATHOLOGY

III. HYPOXYLON CANKER OF POPLAR[1]

by

J. E. BIER[2]

Introduction

It is generally accepted that the economic importance of poplar is increasing, and a more detailed knowledge of the diseases affecting the various species is urgently needed.

This investigation deals with a canker disease found on aspen poplar (*Populus tremuloides* Michx. and *P. grandidentata* Michx.) resulting from the attack of the fungus *Hypoxylon pruinatum* (Klotzsch) Cooke. The heavy mortality due to Hypoxylon canker in widely separated regions clearly indicates this to be a most destructive disease of young aspen. Lorenz and Christensen (15) after making a survey of the disease in the Lake States mention that "*Hypoxylon pruinatum* causes heavy losses throughout the Lake States, and is probably one of the most important diseases of aspen throughout the range of the tree." A major purpose of the study is to describe and illustrate the cankers and causal organism throughout the various stages in their development. Again, it was considered essential to determine, by means of artificial inoculation experiments and observations on the disease in the field, the factors influencing the natural spread of the disease. In the investigation of this phase, experiments are now under way to demonstrate the possibility of the causal organism occurring on hosts other than poplar.

It is felt that studies of this character may assist the forester practising silviculture in aspen stands, with respect to choice of location for permanent sample plots, or the carrying out of improvement cuttings, and in the actual practices of thinning and disposal of diseased material.

Recently poplar-breeding studies have been initiated in Canada. The susceptibility of all parent strains and resulting hybrids to Hypoxylon canker should be determined as a prerequisite to the establishment of plantations.

The Disease

History

Although the causal fungus has been known to occur in North America for over 100 years the first published report connecting *H. pruinatum* with a canker disease of poplar was not made until 1924. In this paper Povah (22) stated that the disease was first observed by Dr. L. H. Pennington in 1920, in Essex county, N.Y. The species *P. tremuloides, P. grandidentata* and *P. tacamahaca* Mill. (balsam poplar) were noticeably affected, the mortality amounting to 27 per cent. A sample plot established at the above-mentioned locality, in a stand containing trembling aspen and balsam poplar showed that 36 per cent of the former were infected, and 26 per cent killed by the disease. Thirteen

[1] This paper constitutes part of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto, April, 1938.

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balsam poplars were present on the plot but only two were infected, and neither killed. Mention is made of a letter received from Mr. J. Elton Lodewick, who found the disease in the area between Orono and Oldtown, Me. In this instance the trembling and large-toothed aspen were commonly infected, but balsam poplar was not found to be diseased. Hypoxylon canker was also found by Povah (22) in Oakland county, Mich., on trembling aspen. Approximately 50 per cent of the trees were infected and 15 to 20 per cent killed. Younger trees appeared to be more susceptible and no cankers were observed on trees with a diameter greater than six inches. A brief description of advanced cankers and the effect of the fungus on the host-bark tissues is presented. Some discussion is given on the synonymy of the causal organism.

In 1925 Schreiner (24) published the results of a preliminary survey of Hypoxylon canker in Maine, and found that in older trees the cankers were, for the most part, located in the upper part of the trunks. In one locality the percentage of infection in trees growing in the open was somewhat higher than in a forest stand.

Faull (10) in 1930 reported the disease in Nova Scotia, and stated that stands are known in which the mortality has amounted to 70 per cent.

Lorenz and Christensen (15) in 1937 reported that the disease caused heavy losses in aspen throughout the Lake States. Aspen, large-toothed aspen, and balsam poplar were apparently equally susceptible. The disease was reported as more prevalent on poor sites, and figures derived from sample plot investigations indicated that trees less than 30 years of age were more heavily infected than older trees. Dominant trees were attacked less frequently than those of the other crown classes.

In 1938, Ponomareff (21) gave a description of the conidial stage of the fungus.

Geographical Distribution

Present knowledge concerning the disease indicates that it occurs only in Canada and the United States. Since its discovery in New York State in 1920, it has been reported from the following regions:—

Canada	*United States*
Nova Scotia	Maine
Quebec	Massachusetts
Ontario	New York
Manitoba	Michigan
Saskatchewan	Wisconsin
Alberta	Minnesota

The approximate location of records from the above listed provinces and states is given in figure 1.

It is important to note that the records available show distribution over a large area of Canada and the northeastern and north-central United States. Reports on the common occurrence of canker in such widely separated regions within a period of 18 years following the first record, strongly indicate that the disease is not of recent origin, but has been present unobserved for many years.

During this investigation the disease has been found in every aspen stand examined in the province of Ontario. Observations were made in the following localities:—

Windsor	Midhurst	Kingston
Chatham	Muskoka	Brockville
London	Temagami	Ottawa
Stratford	Sault Ste. Marie	Arnprior
Guelph	Oshawa	Pembroke
Toronto	Peterborough	Achray

Reference to the map shows that canker is of universal occurrence in the southern portion of the province. Considering this in addition to the previous records, it would seem probable that further studies on distribution will reveal the disease to be present throughout the range of aspen poplar.

FIGURE 1.—Reported Geographical Distribution of Hypoxylon Canker.

OCCURRENCE AND PREVALENCE OF CANKER IN THE FIELD

During the summer of 1938 poplar bluffs in the prairie region of Manitoba and Saskatchewan were inspected for Hypoxylon canker. The disease was present throughout the area, attacking a high percentage of the trees. In certain instances, notably in the Brandon region of Manitoba, well isolated bluffs had been practically eliminated by this disease. The trees killed by canker were producing abundant inoculum capable of spreading the disease to the young reproduction.

The more intense field observations were centred in areas surrounding Toronto, and at the Petawawa Forest Experiment Station, situated approximately 120 miles northwest of Ottawa, Ont.

In the Toronto area most of the work was conducted in a three-acre poplar stand located 20 miles east of the city. The stand was mainly composed of *P. tremuloides* with a few scattered *P. grandidentata* and *P. tacamahaca* along the margins. The tree ages were comparatively uniform, ranging from 10 to 15 years. The results derived from three, one-tenth acre sample plots may serve to illustrate the canker condition of trees in this area. On each plot all standing poplars were tallied and carefully examined for trunk cankers. The numerous branch cankers were not recorded, as in many instances it was not possible to evaluate accurately the extent of their injury to the trees. However, their importance must be considered, as practically all trees had at least one branch infection. Many branches at some previous date had been girdled and killed by cankers located one foot or more out from the main stem (plate II, figure 3). The dead bark in the region between the canker and the main stem was attacked by secondary fungi, and later dried out and separated from the underlying woody tissues. In this way unfavourable conditions were created for the advance of *Hypoxylon* along the dead branch and finally into the living stem. Other branch cankers which occur close to the main stem would undoubtedly spread into the trunks, girdle, and kill the trees (plate II, figure 4). Another

PLATE I *(See opposite page)*

Hypoxylon canker in a 22-year-old stand of aspen, located at the Petawawa Forest Experiment Station, Ont.

Note the spotted character of the diseased areas and their contrast with the healthy bark.

PLATE I (See opposite page)

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important feature of branch cankers is that they frequently produce fruiting bodies of the pathogen which provide a supply of inoculum capable of spreading the disease.

Data obtained from the above mentioned three sample areas are presented in table 1, under Plots 1, 2, and 3. It is evident that from 13 to 25 per cent of

TABLE 1.—INCIDENCE OF HYPOXYLON TRUNK CANKERS IN ASPEN STANDS OF DIFFERENT AGE CLASSES IN ONTARIO

Plot No.	Locality	Age Class	No. of standing trees on plot		No. diseased with trunk cankers		No. killed by trunk cankers		% of Total diseased with or killed by canker	Location of cankers on trunk (Height from ground)
			P. trem.	*P. grand.*	*P. trem.*	*P. grand.*	*P. trem.*	*P. grand.*		
1	Toronto	5–15	190	–	14	–	11	–	13·2	1–20
2	"	5–15	124	–	14	–	12	–	21·0	1–20
3	"	5–15	204	–	34	–	18	–	25·5	1–20
12	Petawawa Forest Exp. Station—	20–25	–	173	–	6	–	1	4·1	3–22
13	"	20–25	13	32	0	0	0	0	13·3	10–32
15	"	30–35	48	18	2	2	0	0	6·1	36–50
17	"	30–35	26	43	1	1	3	0	7·2	16–30
7	"	60–65	–	24	–	3	–	0	11·1	56–65
11	"	60–65	12	1	1	0	0	0	7·7	60–67

the standing trees were either killed by canker, or were so seriously infected that they would succumb within a few years. Although these percentages are smaller than those given by some investigators, they still represent a substantial proportion of the stand.

It is essential to point out that in a 10- to 15-year-old stand of poplar, although actually 13 to 25 per cent of the trees may be killed by cankers, it must be borne in mind that at this age the stand is very dense, and the number of trees would be considerably reduced by natural competition if the disease were not present. Therefore, primary interest lies in the effect of the disease on trees that will form a part of the final crop. The suggestion has been made that Hypoxylon canker is beneficial, for the reason that it acts as an aid to natural thinning in younger stands. This perhaps would be true if the disease occurred only in the form of trunk cankers on suppressed or otherwise weakened trees. Field observations, confirmed by inoculation experiments, repeatedly demonstrate that the faster growing dominants and suppressed trees are equally susceptible. This fact is illustrated to advantage in the sample plot investigations, by tabulating infected trees according to diameter classes as shown in table 2.

TABLE 2.—TRUNK CANKERED TREES ON 3 SAMPLE PLOTS ESTABLISHED IN A 10-15-YEAR-OLD ASPEN STAND TABULATED ACCORDING TO DIAMETER CLASSES

D.B.H. (Inches)	Total Number of trees on			Number of trees with trunk cankers on			Percentage of total with trunk cankers on		
	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
1	72	58	132	9	16	31	13	28	24
2	61	33	56	5	3	14	8	9	25
3	37	16	15	6	2	6	16	13	40
4	12	12	1	2	3	1	17	25	100
5	6	3	–	3	1	–	50	33	–
6	2	2	–	–	1	–	0	50	–
7	–	1	–	–	–	–	–	0	–

From the table it is evident that trees bearing trunk cankers occur throughout the diameter classes represented on the plots. In many instances the percentage of diseased trees is higher in the larger diameter classes, which include trees that undoubtedly, if not diseased, would form part of the final crop.

To summarize, the importance of Hypoxylon canker in younger aspen stands is dependent upon the following considerations:—

(1) The presence of numerous cankers killing individual branches.
(2) The spread of branch cankers into the trunks, resulting in the death of the trees.
(3) The large number of dominant as well as suppressed trees killed by trunk cankers.
(4) The abundant production of spores on branch and trunk cankers, which disseminate the fungus and spread the disease to healthy, fast-growing trees which should form an important part of the final crop.

During the summers of 1935 and 1936 poplar heartrot investigations were conducted at the Petawawa Forest Experiment Station. One-tenth-acre sample plots were established in stands from 20 to 65 years of age. As all poplars on the plots were felled, it was possible to examine the entire bole of the largest trees for the presence of canker.

The results obtained from these plots are included in table 1. Branch cankers occurred on trees of all ages, and on several occasions viable fruiting bodies were found on branch lesions located from 70 to 80 feet above the ground level. The table shows that trunk cankers are present on trees from 20 to 65 years of age. Another feature not shown in the sample areas studied near Toronto is the incidence of the disease on *P. grandidentata* as well as *P. tremuloides*. It is important to observe the position of trunk cankers on the older trees. As the trees become older the cankers are found higher on the trunks until in the 65-year-old trees the infections occur from 56 to 65 feet above the ground level. It would appear that it is not the age of the tree which is the limiting factor in susceptibility to canker, but rather the age of the bark. The fungus in a parasitic capacity is found growing throughout the thick green cortex present in young poplar bark. In older bark this cortex is replaced by resistant cork tissue formed from the successive cork cambium layers produced as the result of wounding and lateral trunk expansion. The smaller number of cankers at the higher elevation is possibly correlated with sparser inoculum at these altitudes, plus a less frequent occurrence of environmental conditions conducive to infection.

Hosts

Hypoxylon canker is commonly found on *P. tremuloides* and *P. grandidentata* in Ontario. Several stands of *P. tacamahaca* have been examined, but no living cankered trees observed. At Toronto in 1934 inoculations were made on balsam poplar using the identical methods employed to obtain positive results on aspen. They all proved negative. In 1936 inoculations were made on balsam poplar at the Petawawa Forest Experiment Station. Examination one year later demonstrated all inoculations to be negative. Balsam poplars of the same plantation inoculated with two strains of *Cytospora* resulted in canker production, thus apparently eliminating the possibility of improper technique.

The first incidence of Hypoxylon canker on balsam poplar was observed in Saskatchewan and Alberta during 1938. The trees were growing on a poor, dry site, and were infested with bark-boring beetles, conditions which may have increased their susceptibility. At present no definite explanation is advanced for the apparent absence of the disease on balsam poplar in Ontario. The

PLATE II *(See opposite page)*

FIGURE 1. Retention of the dead leaves on trees killed by trunk cankers during the growing season.

The trees were killed during the summer of 1934 and photographed in April, 1935. The tree on the right has broken off at the position of the canker.

FIGURE 2. Branch flag; the branch was killed by the advancing trunk canker.

FIGURE 3. Branch killed by the canker shown as the slightly swollen area at approximately the mid-point along the branch.

FIGURE 4. Trunk canker resulting from the continuation of a branch canker into the main stem.

PLATE II (See opposite page)

PLATE III *(See opposite page)*

Incipient Hypoxylon cankers demonstrating the mode of infection.

Observe the irregular, lobed, advancing margins of the diseased areas. X 0.5.

FIGURE 1. Branch node infection on *P. grandidentata.*

Note the slightly depressed area of diseased bark in advance of the canker body.

FIGURE 2. Canker located at the base of a dead branch on *P. tremuloides.*

Note the dark patch, "conidial blister", surrounded by diseased bark at approximately the central position of the canker.

FIGURE 3. A lesion centred around the wound made by a wood-boring beetle of the genus *Oberea.*

Observe the mottled character of the diseased bark.

FIGURE 4. Longitudinal cross-section of Figure 3.

Note the borer tunnel extending along the pith region and then directly to the outside.

FIGURE 5. Canker associated with an undetermined form of insect injury.

FIGURE 6. Longitudinal cross-section of Figure 5.

Observe the insect tunnels, and the blackened diseased bark tissues.

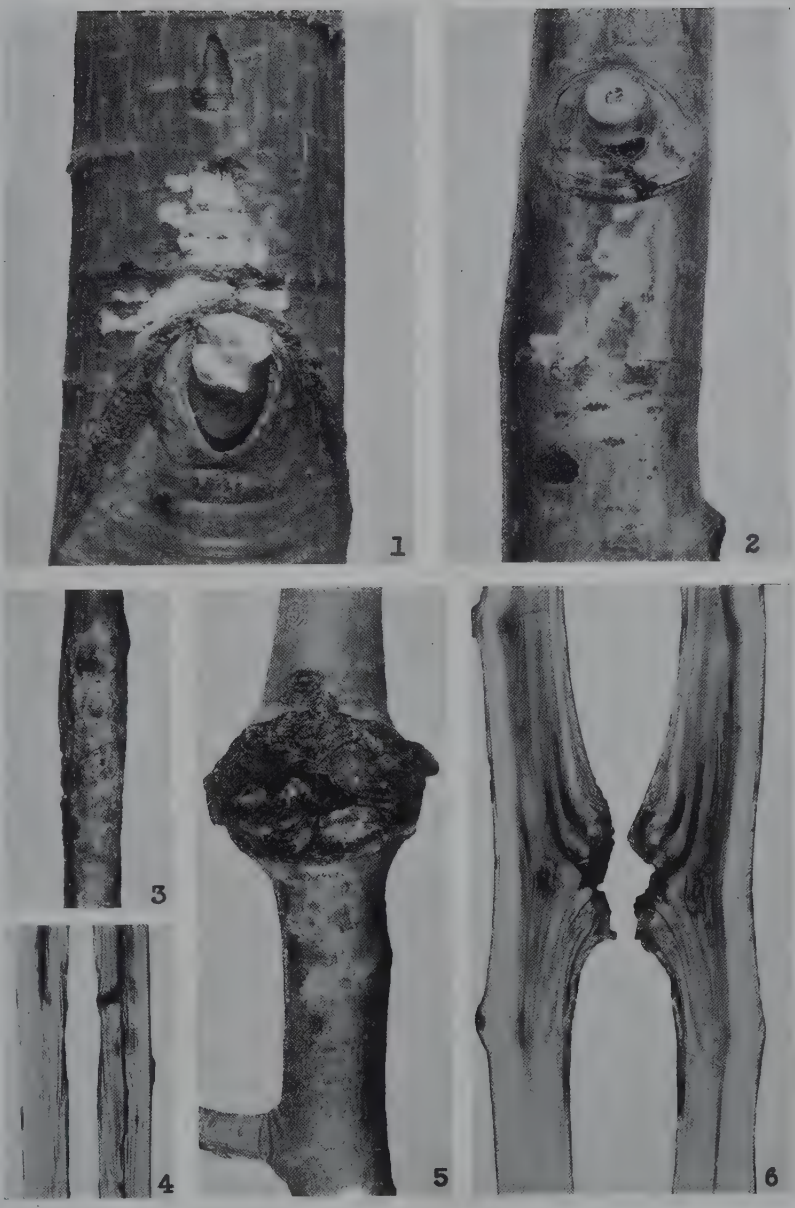

PLATE III (See opposite page)

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possibility of different host strains, and lowering of host resistance by adverse environmental conditions, may be cited as factors to account for the susceptibility of this species in the Prairie Provinces.

In natural aspen stands one frequently finds one or more of the species *Alnus, Betula, Acer, Quercus,* and *Salix,* growing in association with the poplar. Forms of *Hypoxylon* commonly occur on dead portions of the above trees. Identification of the fungi places the fungus on alder in the species *H. Morsei* B. and C.[1], the birch in *H. pruinatum*[1], and the forms on maple, oak, and willow in *H. Blakei* B and C.[1] Previous mycological investigations have shown that these three species are closely related, and some authorities combine them as one. During the summer of 1937 the fungi from the above hosts were inoculated on poplar. These parasitism studies may assist in determining whether the above forms are the same as the canker-producing fungus on poplar. If such proves to be the case, their great importance in adding to the amount of inoculum may readily be realized. Further, these host species, as well as poplar, would have to be duly considered before advocating control measures.

Mode of Infection

Artificial inoculations and field observations of incipient natural cankers convincingly demonstrate that the fungus is a wound parasite.

Bark punctures resulting from insect attack commonly serve as centres of infection. In the vicinity of Toronto the most prevalent type of insect injury associated with cankers is caused by a species of *Oberea,* a wood-boring beetle of the group Cerambycideae. The healthy bark of young stems and branches is punctured by this insect in the process of oviposition. The larvae hatch and bore into the pith region where they tunnel up or down producing frequent ventilation tunnels to the outside as they advance. After boring in the wood for approximately two years, pupation occurs and the adult beetles emerge. Practically all aspen trees in this region are attacked by this insect and Hypoxylon cankers are centred around the entrance, ventilation, and emergence tunnels (plate III, figures 3 and 4). Cankers of this type have been collected when the larvae are still in the host. Plate III, figures 5 and 6, shows a canker extending out from another undetermined form of insect injury. The poplar borer (*Saperda calcarata* Say) also makes openings in the healthy bark of aspen trunks, but cankers have not been found associated with this type of wound. A heavy flow of brownish sap exudes from the tunnels, and may be instrumental in preventing infection.

Another common avenue of entrance is the branch node. In some instances cankered bark is present on the dead branch, indicating the continuation of a branch canker into the main stem (plate II, figure 4). In others there is no evidence of the disease on the lateral branch, suggesting that the fungus has grown saprophytically along the dead branch into the main stem, again becoming parasitic and initiating canker formation (plate III, figures 1 and 2).

Cankers are also found associated with mechanical wounds, for example, axe cuts and breakages caused by wind.

Symptons

In aspen stands up to 30 years of age the trunk- and branch-cankered trees with roughened, spotted grey and black bark, stand out in decided contrast to the smooth, greenish-grey bark of healthy trees (plate I). A brownish sap flow frequently occurs at the canker margins.

[1] Dr. C. L. Shear kindly confirmed the author's identification of these fungi.

Trees girdled and killed by canker, and living diseased trees, break off at the position of cankers, and it is common to find trees broken at various heights above the ground level (plate II, figure 1). The wood underlying a cankered region is considerably weakened by the action of wood-destroying fungi and the tunnels produced by secondary bark and wood-boring beetles.

Abnormally small, slightly yellowish leaves are produced on trees and branches seriously affected by canker, and those killed during the summer retain their foliage until the following spring. The trees shown in plate II, figure 1, were killed during the growing season of 1934, and photographed in April, 1935. Plate II, figures 2 and 3 illustrate the "flags" resulting from the killing of individual branches.

Incipient cankers are observed as yellow to reddish-brown areas with irregular, lobed, advancing margins, always centred around some form of wound (plate III). Discoloured, slightly depressed patches of diseased bark are often found in advance of the main body of the canker and separated from it by green cortical tissue (plate III, figure 1). As the cankers increase in size, the affected bark takes on a distinctly mottled appearance, owing to the segregation of the blackened diseased cortical tissue into irregularly shaped areas by yellowish lines and patches (plate III, figure 3). By the end of the first year, or during the second, the unbroken periderm is raised in spots by the formation of the conidal fructifications. The periderm later ruptures, exposing the greyish conidial layer (plate IV). In plate III, figure 2, the dark patch surrounded by yellow diseased bark at about the central position on the canker, illustrates a "conidial blister" on a young infection. At the time the specimen was collected, the periderm was pushed out from the underlying cortex, and when broken demonstrated the conidial layer. The host frequently produces a callous layer separating diseased from healthy bark. This results in a vertical cracking of the healthy bark along the canker margins, and leaves the diseased bark in a slight depression. In most instances the fungus is able to overcome this protective layer, and in older cankers it is often possible to see three to four annual zones of callous tissue. During the second and succeeding years the diseased bark is attacked by the larvae of secondary bark-boring beetles. These insects concentrate on infected trees and the infestation becomes very heavy in the diseased bark of older cankers. The advanced cankers have a spotted appearance, the greyish to yellowish flaking periderm contrasting with the blackened insect-emergence tunnels and old conidial fructifications (plate I, and plate IX, figure 3). The advancing margins remain irregular and wavy, and of a yellow to reddish-brown colour.

Between the second and third years the first immature perithecial stromata are usually visible on the cankers. This is a most critical point, as the trunk cankers are well advanced, in most cases two or three feet long before the perfect fruiting bodies become apparent. Artificial inoculations and young cankers under continuous observation since May, 1934, demonstrated the first signs of perithecia in October, 1936. By ring counts, the age was determined for a number of trunk cankers producing their first perithecia and all were three or more years of age. The greyish to black perithecial stromata are found abundantly on the older cankers (plate V, figures 1 and 2). As a general rule trunk cankers are three or more feet long before the trees are girdled and killed.

If the diseased bark near the margin of a canker is removed with a knife it is possible to see fans of fungous mycelium in the bark and cambium tissues. The growing margin of the fans is white, while farther back the hyphae turn a dark greenish colour. Microscopic examination of sections cut through the diseased bark shows the fungus to be present in the cortex, phloem, cambium, and recently formed xylem elements whose walls are not fully lignified. The fungus is typically a bark parasite and has not been found to attack the wood for any appreciable distance beneath the cambium. The hyphae penetrate the

PLATE IV *(See opposite page)*

Imperfect stage of *H. pruinatum.*

FIGURE 1. Imperfect fructifications produced in the form of "conidial blisters" on the diseased bark of a 2-year-old canker. X 0·5

Observe the unbroken and partly broken "conidial blisters" along the right hand side of the canker, and the large open blister shown near the bottom of the photograph.

FIGURE 2. An enlarged view of a single open "conidial blister". X 6

Note the erect, pillar-like structures which force the periderm out from the underlying tissues and the greyish conidial layer covering the surface of the diseased cortex.

FIGURE 3. Longitudinal section through an unbroken "conidial blister". X 13

Note the hyphal, pillar-like structures forcing the periderm out from the underlying tissues, and the conidial layer covering the sides of the pillars and the outer surface of the diseased cortex.

FIGURE 4. Longitudinal section through a single hyphal pillar. X 90

Note the top of the pillar pressed tightly against the periderm, and the darkened layer of conidia outside the lighter layer of conidiophores which cover the sides of the pillar and surface of the diseased cortex.

FIGURE 5. Conidia produced in a hanging-drop culture. X 250

Observe the branched conidiophore, and the oblong to ovoid, one-celled conidia. The spore at first borne apically becomes lateral by sympodial growth of the hypha, and another spore is formed. This process is repeated in a manner such that heads of spores are produced on the ends of the conidiophores.

FIGURE 6. Camera-lucida drawings of conidia produced in nature. X 1000

Note the variation in the size of the spores.

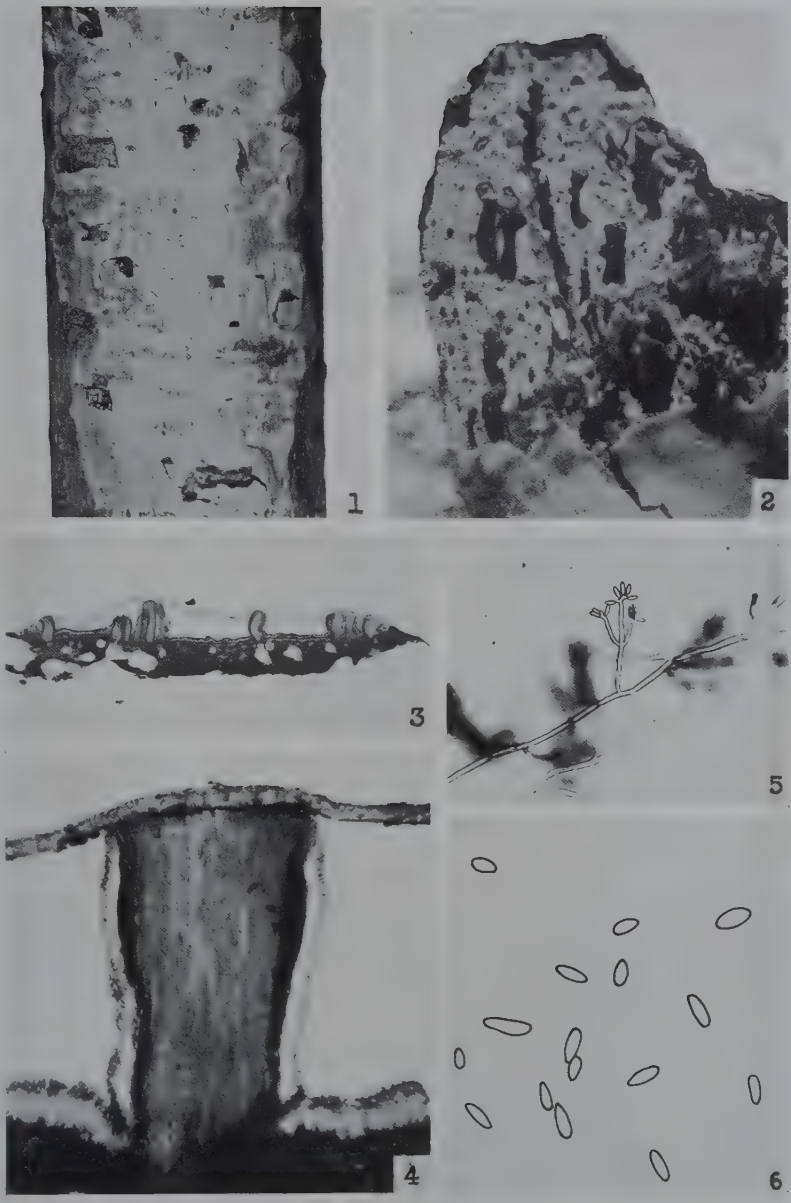


PLATE IV (See opposite page)

cell walls, later entirely filling the cells with mycelium and causing complete disintegration. The sclerenchyma and cork cells with thick, lignified and suberized walls are not attacked. The hyphae later turn dark and the diseased bark appears as a solid, black, fungous stroma, with the lighter sclerenchyma and cork tissue scattered throughout.

The Causal Fungus

Taxonomy

The fungus associated with cankered poplar trees in Ontario agrees with the description of *Hypoxylon pruinatum* given by Ellis and Everhart (8), and Specimen *1182* in Ellis, North American Fungi.

The fungus was first described by Klotzsch (13) in 1833 as *Sphaeria pruinata*. The description was based on a North American collection made by Dr. J. Richardson. Currey (6) in 1858 describes *S. pruinata* Kl. in his synopsis of the compound *Sphaeriae* of the Hookerian Herbarium. Saccardo (23) in 1882 transferred the fungus to the genus *Rosellinia*, calling it *R. pruinata* (Kl.) Sacc. Cooke (4) in 1883, reviewing Saccardo's species of *Hypoxylon*, transfers the fungus to the genus *Hypoxylon*. In doing so he states the fungus to be "An undoubted *Hypoxylon*, in which relationship it was placed by Klotzsch." Since Cooke transferred the fungus to *Hypoxylon* it should be considered *H. pruinatum* (Kl.) Cke. Ellis (7) in 1883 described a fungus on poplar from Iowa as *H. Holwayii*. Cooke (5) in 1884, Ellis and Everhart (8) in 1892, and Povah (22) in 1924 consider *H. Holwayii* Ellis a synonym of *H. pruinatum*. The only difference in the descriptions of the two species is the presence of projecting, bristle-like teeth from the bark surface surrounding the perithecial stromata of *H. Holwayii*. This investigation also indicates that the two species are synonymous, and evidence is presented in the section dealing with the morphology of the imperfect stage to show that the projecting teeth are the remains of old conidial fructifications, formed a year or two previous to the perithecial stromata.

From the above discussion the fungus is *Hypoxylon pruinatum* (Kl.) Cke., with the following synonymy:—

Sphaeria pruinata Klotzsch
Rosellinia pruinata (Kl.) Saccardo
Hypoxylon Holwayii Ellis

Morphology

The Imperfect Stage

During May and June 1934, conidia produced on cankers were cultured and the mycelium from these isolations utilized for the inoculation of 35 healthy aspen trees. The inoculations were uniformly positive and the imperfect stage was evident on the induced cankers as early as five months after inoculation. Some cankers did not produce the imperfect stage until July, 1935, or 14 months after inoculation. Natural cankers were found to produce conidia at the end of the first or during their second growing season. The fruiting bodies have a characteristic structure and essentially agree with the description given by Ponomareff (21).

The fructifications appear primarily as blisters of various sizes on diseased poplar bark (plate IV, figure 1). Sections through the blisters indicate that the fungus produces hyphal pegs or pillar-like structures that force the superficial periderm out from the underlying cortical tissue (plate IV, figure 3). The outer surface of the cortex and sides of the pillars are solidly covered by a greyish layer of conidiophores and conidia (plate IV, figure 4). The periderm

finally ruptures as the result of the upward pressure exerted by the growing pillars, and the conidial layer is exposed (plate IV, figure 2). The open blisters dry out and the conidial layer exposed to the weather soon disappears, leaving the dried, shrunken pillars projecting from the blackened cortex. The imperfect stage is produced on a canker one or two years previous to the first formed perithecia. A cankered area with mature perithecia, except for the projecting teeth, has usually lost all signs of the conidial stage. In advanced cankers it is common to find perithecia on the older diseased bark and fresh conidial fructifications nearer the canker margins (plate IX, figure 7).

The conidia are one-celled, hyaline or brownish in mass, borne on brownish, branched, septate conidiophores with a diameter ranging from 2 to 4 microns. The spore borne apically later becomes lateral by sympodial growth of the hypha, which produces another spore and leaves the first on a shoulder-like joint. In many instances this phenomenon continues in such a manner that heads of spores are formed on the ends of the conidiophores (plate IV, figure 5). The spores are oblong to ovoid, measuring from 4·5 by 1·2 to 7·0 by 2·5 microns (plate IV, figure 6). The conidiophores and conidia produced in culture are essentially the same as those found in nature.

At this point reference is made to Povah's (22) discussion of *H. pruinatum*, which includes a citation from Ellis and Everhart (8):—

> "According to these authors, *H. Holwayii* differs from *H. pruinatum* in that 'surrounding the stroma and standing out obliquely like a coarse fringe, are short, coarse, black, bristle-like teeth, like the teeth of a *Hydnum* or *Irpex*. This curious growth also arises from the surface of the inner bark for some distance around the stroma, soon throwing off the epidermis and leaving the blackened surface of the inner bark exposed.'
>
> In our specimens the bristly appearance is found commonly but not constantly. In some cases it is due to the presence of small perithecia protruding from the inner bark in which they are formed, little or no stroma being present. Sometimes the spine-like appearance is obtained by the breaking down of the perithecia leaving the jagged remnants of stroma and perithecial walls. In still other cases the dark teeth proved to be the more persistent parts of the inner bark left projecting after the softer part has been decayed."

It is suggested that the dark, spine-like teeth often found associated with the perithecial stromata by the above authors, are the dried pillars, the only remaining part of old conidial fructifications. Careful observation of the location of perithecia on natural and artificial cankers conclusively demonstrates that perithecia may occur at some distance from the nearest "conidial blister." In a collection these stromata would not show the associated projecting teeth, and consequently the bristly appearance referred to above would not be found constantly.

The Perfect Stage

Hypoxylon cankers as a rule are three or more years of age before the appearance of the first perithecial stromata. Since the conidial stage is usually produced abundantly, and "conidial blisters" cover large areas of the diseased bark, the perithecia frequently break through the cortex at a position formerly occupied by the conidial stage. In these instances the cortex immediately surrounding the stromata is covered with the black, hyphal pillars remaining from the imperfect fructifications.

The erumpent stromata vary a great deal in shape and size (plate V, figure 1). Therefore, the number of perithecia in a single stroma is also quite

PLATE V *(See opposite page)*

Perfect stage of *H. pruinatum.*

FIGURE 1. Perithecial stromata on a 5-year-old canker. X 1

Observe the variation in the shape and size of the stromata.

FIGURE 2. Perithecial stromata enlarged. X 6

The erumpent stromata except for the projecting ostioles are more or less flattened and covered with a white, pruinose material.

FIGURE 3. Cross section of a perithecial stroma. X 19

The single-layered perithecia are covered by a hard, black, ectostroma, and seated directly in the bark tissues. Note the sclerenchyma bundle in the area separating the 2 perithecia on the right. The asci and filiform paraphyses are borne along the base and sides of the perithecia.

FIGURE 4. Photomicrograph of a single ascus. X 320

The asci are cylindrical, and the brown, one-celled, ascospores are borne uniseriately in the ascus.

FIGURE 5. Photomicrograph of the oblong to ellipsoid ascospores, the 2 lower showing the elongate germination slit present in the spores. X 760

FIGURE 6. Photomicrograph of a germinating ascopore, illustrating the germ tube extending out of the germination slit for the entire length of the spore. X 520

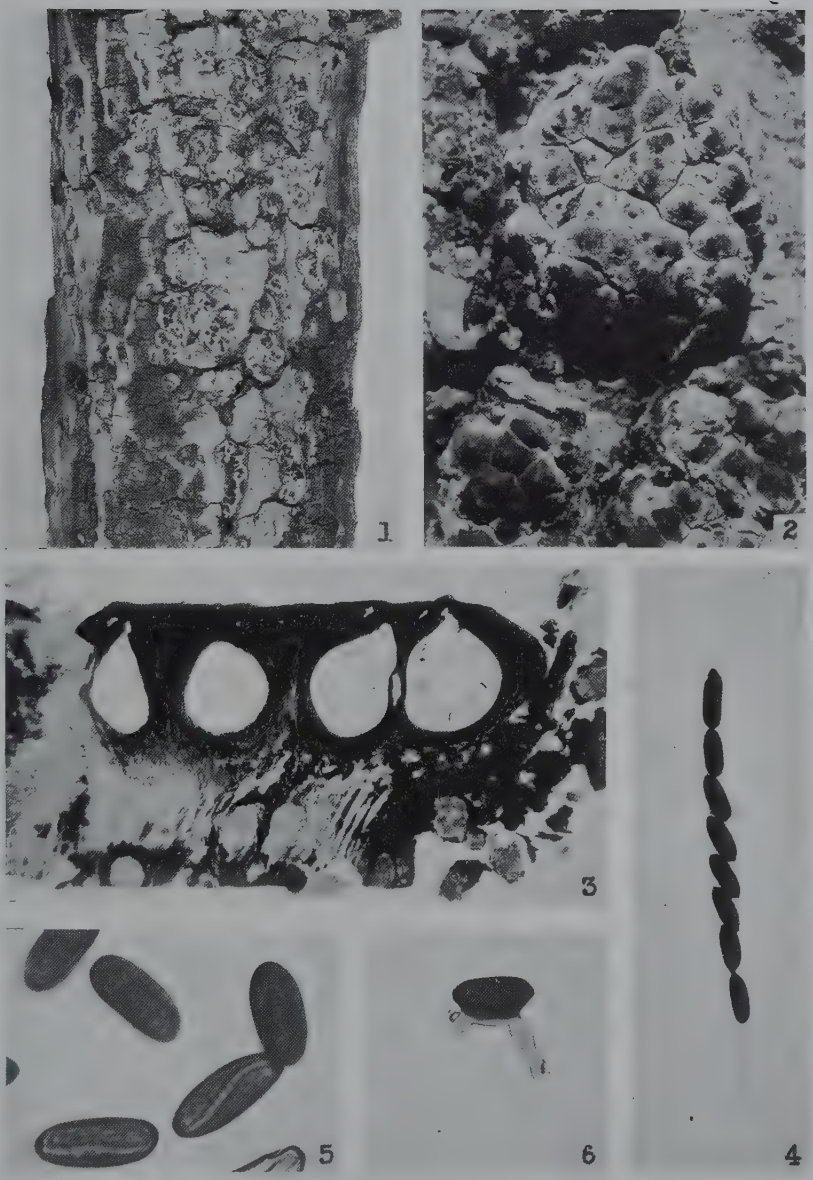


PLATE V (See opposite page)

PLATE VI *(See opposite page)*

FIGURE 1. Photomicrograph of a germinating condium 4 days after it was placed in a hanging drop culture. X 250

The spore has swollen considerably and lost its oblong form.

FIGURE 2. Same spore as illustrated in Fig. 1, 24 hours later. X 250

Observe how the original spore body has collapsed, leaving granular, protoplasmic material in the surrounding area.

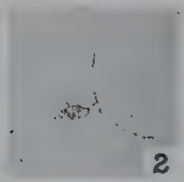
FIGURE 3. Photomicrographs of 2 germinating conidia 12 days after they were placed in a hanging-drop culture. X 250

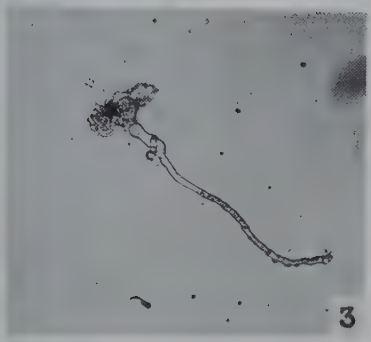
One spore has produced a stout germ tube of a much greater diameter than the originals. These on occasion continue to grow and finally form a mycelial colony.

FIGURE 4. Photograph demonstrating the construction of the spore traps employed in the field studies on ascospore discharge.

FIGURE 5. The photograph illustrates ascospore deposits of *H. pruinatum* on microscope slides. Slides numbered from 1 to 8, and 9 to 16, show the discharge obtained in traps established on trees 1 and 2 respectively. X 0·5.

Observe that slides 1 and 9, although exposed for an 8 hour rain, show no macroscopically visible ascospore deposits. Slides 2 to 6 and 10 to 14 demonstrate the very heavy discharge the first hours after the rain and the gradual diminution to the 25th hour, after which the discharge is very meagre. Numbers 8 and 16 illustrate that no discharge occurred later than 13 hours after the thundershower.





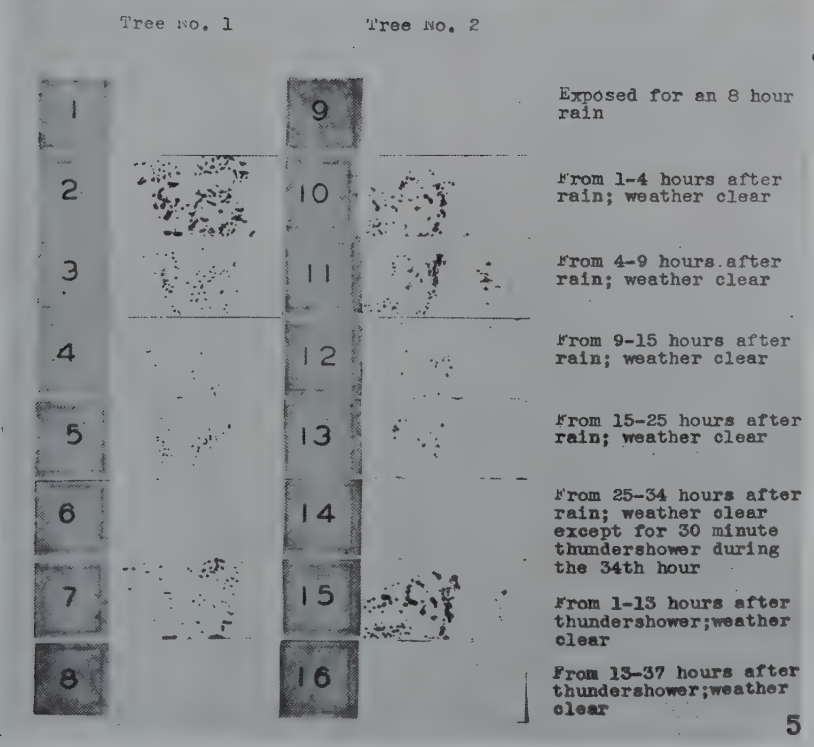


PLATE VI (See opposite page)

variable. The outer surfaces of recently matured stromata are more or less flattened, and except for the projecting ostioles, are covered by a white pruinose coat (plate V, figure 2). This later disappears and the stromata appear black throughout.

Longitudinal sections through a stroma show that the perithecia are covered by a hard, black ectostroma. The perithecia occur in a single layer, seated directly in the diseased bark, and not surrounded by an entostroma (plate V, figure 3). The bases and walls of the perithecia are lined with cylindrical asci and filiform paraphyses. The asci range from 160 to 190 microns long (spore part), with a stalk from 40 to 60 microns long. The one-celled, brown, oblong to elliptical ascospores are borne uniseriately in the ascus, and measure from 24 to 30 by 9 to 13, with an average of 26 by 11 microns (plate V, figure 4). One, or frequently two, large oil globules are usually visible in the spores and germination occurs through an elongate slit along one side of the spore (plate V, figures 5 and 6).

Studies on Spore Germination

Conidia

Conidia were scraped from the surface of recently broken "conidial blisters," and placed in sterile water. Hanging-drop cultures were prepared by placing a drop of the conidal suspension on the under surface of cover slips thinly coated with potato dextrose or malt agar.

Within 48 hours single isolated conidia had swollen to several times their normal size and germinated. After five to seven days the germ tubes stopped growing in most of the spores and the original spore bodies collapsed, leaving a mass of loose, granular, protoplasmic material in the surrounding area (plate VI, figures 1 and 2). Many spores were examined for several days following the time of collapse but no further growth occurred. A small number of spores about eight days after germination produced stout germ tubes of a much larger diameter than the originals (plate VI, figure 3). Some of these continued to grow and produced many branch hyphae which later formed a colony of mycelium.

Groups of germinating conidia continue growth and finally produce mycelial colonies.

Ascospores

Ascospores discharged on the surface of potato dextrose or malt agars germinate within 24 hours. Practically all the spores germinate, and each is capable of growing and producing a colony of mycelium. A majority of the spores produce two germ tubes, one at each end of the spore (plate V, figure 6).

Ascospores obtained from perithecia stored in the laboratory for a period of six months germinated and grew equally as well as material recently collected from the field.

Cultural Studies

Conidia

Water suspensions of conidia produced in nature and in cultures were poured into Petri dishes containing malt agar. In both instances many colonies of the *Hypoxylon* fungus were obtained. The conidia produced in nature germinated and formed colonies initially comprised of white mycelium of the same character as those derived from germinating ascospores (plate VII, figure 12). More than a month passed before conidia were produced on these cultures. Usually the conidia derived from cultures developed into colonies that immediately produced more conidia and the entire surface of the colonies became covered with spores (plate VII, figure 9). Occasionally conidia derived from cultures formed white mycelial colonies similar to those obtained from germinating ascospores. Six colonies resulting from the germination of conidia produced in an ascospore culture are shown in plate VII, figures 10 and 11, and

the range in variation is evident from a white mycelial colony to those completely covered by spores.

Ascospores

Single ascospores were isolated and differences observed in the appearance of the colonies formed on potato dextrose agar. The 16 spores were then isolated from two asci occurring in perithecia of two different collections. On potato dextrose agar the eight spores of each ascus produced colonies that fell into four morphologically distinct groups. Plate VII, figures 1, 2, 3, and 4, illustrates the differences found in one ascus. The spores were numbered arbitrarily, and therefore the spore number does not indicate its position in the ascus. It is evident that the colonies resulting from mycelial transfers of cultures derived from spores 1 and 2 appear identical (plate VII, figure 1), and the same is true of spores 3 and 4 (figure 2), 5 and 6 (figure 3), and 7 and 8 (figure 4). Further, the appearance of each group of two spores producing identical colonies is different from the other three groups. Although the photographs illustrate colonies formed when two spore transfers are growing in one Petri dish, the same results are derived when mycelium from each spore is plated out singly. After four to six weeks' growth, a more or less solid black stromatic layer was produced under the aerial mycelium of colonies from spores 1 and 2 (plate VII, figure 1), and 5 and 6 (figure 3), while very little stromatic material was present in the other four (figures 2 and 4). At this time greyish to brownish patches comprised of conidiophores and conidia appear scattered over the surface of the colonies.

Interesting results were obtained when mycelial transfers of cultures derived from the eight spores of a single ascus, were paired in all possible combinations. The colonies grew together in only four of the 27 pairings. The remaining 23 produced a distinct line of demarcation between the two colonies. Pairings involving two spores producing identical colonies grew together (plate VII, figures 1, 2, 3, and 4). Pairings between spores 1 and 3 (figure 5), 2 and 4 (figure 6), 5 and 7 (figure 7), and 6 and 8 (figure 8), serve to demonstrate the line of demarcation formed in the remaining pairings. The colonies grew together in all selfed pairings.

The above experiment was repeated using the eight spores of an ascus derived from a different perithecial collection. Identical results were obtained with the exception that the four morphologically similar groups in this instance did not appear the same as those of the first ascus.

Cultures obtained from the germination of several ascospores were of the same character as those derived from conidia and single ascospores. A number of small, globose, stroma-like bodies were formed on the agar at locations where a number of spores had germinated and grown together. This may have been due to the production of numerous lines of demarcation isolating small colonies, and in this way prohibiting further horizontal expansion.

Several trials have been made to obtain the perithecial stage in pure culture, but to the present all have proved negative. The following materials have been placed in flasks containing small amounts of water or agar, and inoculated with mycelium from single and mixed ascospore cultures.

(1) Poplar twigs, steam and surface sterilized.
(2) Poplar bark, steam and surface sterilized.
(3) Diseased poplar bark from cankered regions producing perithecia, steam sterilized.
(4) Alder twigs, steam and surface sterilized.

In most instances a mat of mycelium one-eighth of an inch or more thick was formed over the bark and wood surfaces.

On many occasions pure cultures of the fungus *H. pruinatum* have been obtained from diseased bark isolations made at the margins of natural and induced cankers.

PLATE VII *(See opposite page)*

Cultural studies of *H. pruinatum.* X 0·5

FIGURES 1 to 4. Three-week-old colonies on potato dextrose agar resulting from mycelial transfers of cultures derived from the 8 ascospores of a single ascus.

The spores were arbitrarly numbered from 1 to 8, and 2 isolates are present in each Petri dish. The colonies from spores 1 and 2 (Figure 1) are essentially alike, and the same is true of spores 3 and 4 (Figure 2), 5 and 6 (Figure 3), and 7 and 8 (Figure 4). The appearance of each group of 2 spores producing similar colonies is different from the other 3 groups. When mycelium of the 8 spores is paired in all possible combinations, fusion occurs in only 4 of the 27 pairings. The colonies grew together when 2 spores producing morphologically similar colonies were plated together.

FIGURES 5 to 8. Demonstrates the line of demarcation produced in all remaining pairings.

Figure 5—spores 1 and 3
Figure 6—spores 4 and 2
Figure 7—spores 5 and 7
Figure 8—spores 8 and 6

FIGURE 9. Colonies present 16 days after the application of water suspension of conidia produced in an ascospore culture.

Additional conidia are immediately produced and the colonies become completely covered with spores.

FIGURES 10 and 11. Six colonies, 21 days old, resulting from the germination of conidia which were produced in an ascospore culture.

Note variation from a colony consisting of white mycelium to those entirely covered with conidia.

FIGURE 12. Colonies present 16 days after the application of a water suspension of conidia produced in nature.

Note that the colonies are comprised of white mycelium which is identical with that obtained from germinating ascospores.

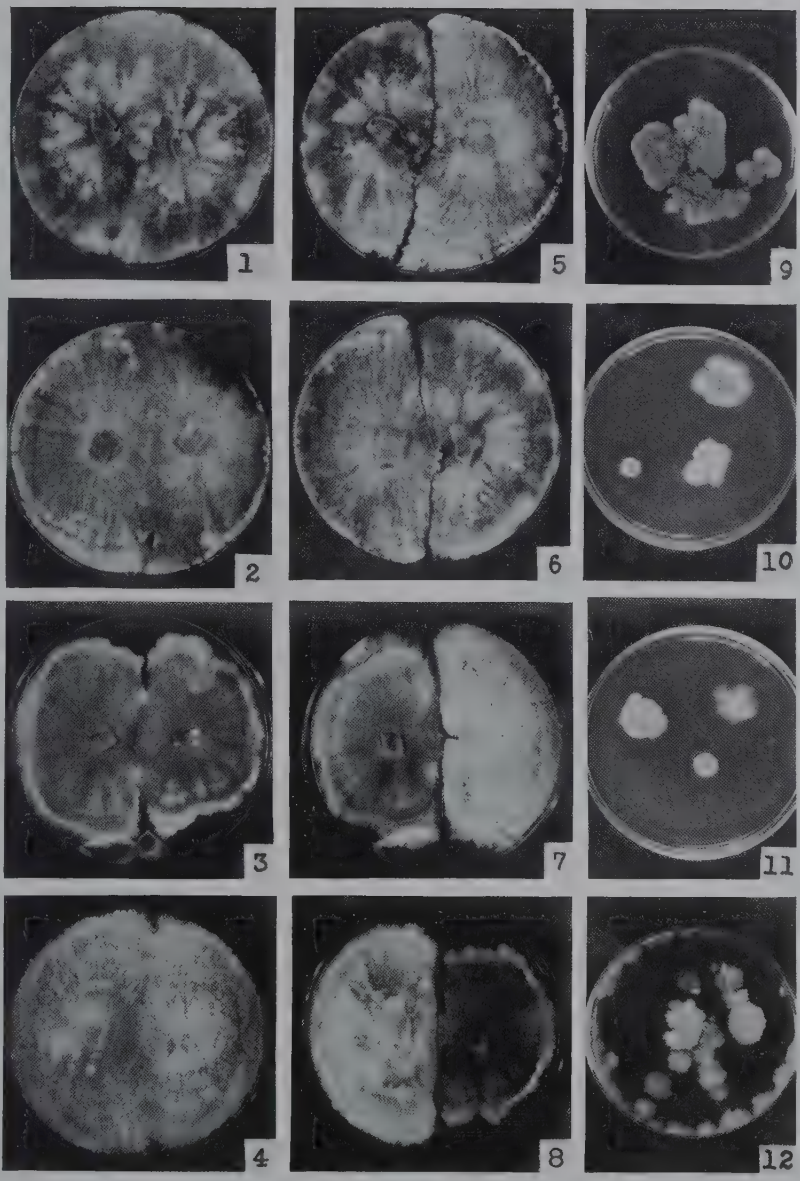


PLATE VII (See opposite page)

Spore Discharge Studies

Laboratory Experiments

Ascospore discharge may be observed with the naked eye when a strong beam of light in an otherwise darkened room is placed at a level just above the ostioles of moistened, viable perithecia. The asci are discharged in rapid succession from a perithecium, the eight spores travelling vertically in a row to a distance of from two to four centimetres above the ostiole.

Discharge was also studied under a binocular microscope. In most instances, a single ascus would pass through the ostiole at one time, although occasionally the spores from two asci were discharged simultaneously. In six trials it was determined that from 45 to 61 asci were discharged from an ostiole in one minute. The spores were discharged after the top of the ascus and the uppermost one or two spores had passed through the ostiole. This phenomenon was very rapid and could be observed to the best advantage when the perithecia were placed so that the emerging asci could be seen horizontally. At rather frequent intervals an ascus would pass through the ostiole intact. If these asci are not washed away from a perithecium which has been discharging spores for some time, they remain in the form of a black mound surrounding the ostiole.

Spores discharged from individual asci were examined as they reached a microscope slide placed four millimetres above the ostiole of a perithecium. The eight spores from an ascus appeared to strike the slide at about the same time. Invariably four, five, or six of the spores would lie side by side in a row, and the remainder in groups or singly, at some distance from the main group.

When actively discharging perithecia are placed under water, the asci pass through the ostioles much more slowly than they do in air. The spores remain in the ascus, and intact asci may be observed floating on the surface of the water. Apparently the difference in pressure between air and water is sufficient to prevent the asci from bursting. Material under water for some time discharged normally when placed back in the air.

Viable perithecia in a dried condition were suspended in an atmosphere saturated with water vapour to determine whether spore discharge occurs when they are not in direct contact with water. In 24 hours discharge was evident for a species of *Rosellinia* growing superficially on the bark, but no *Hypoxylon* spores were present. Examination on the fifth day following the initiation of the experiment revealed that discharge had taken place in each of six established trials.

Tests were carried out to discover the maximum distance ascospores are shot vertically. In five trials at room temperature it was found that a few spores were caught on slides placed 45 millimetres above the ostioles. The spores were well scattered, singly, or in groups of two, appearing as though only one or two spores from an ascus were reaching the slide. Heavy discharge occurred from 20 to 25 millimetres above the ostioles and groups of four or six spores in rows were common, suggesting that all the spores from an ascus were reaching the slides.

Field Experiments

During the summer of 1936 at the Petawawa Forest Experiment Station, two spore traps were established to determine the incidence and length of the discharge period for perithecia occurring under field conditions. One trap was located on a living diseased *P. grandidentata* (tree No. 1), and the other on a dead standing *P. tremuloides* (tree No. 2). The construction of the spore traps consisted of suspending microscope slides two to three millimetres away from groups of viable perithecia. A protective covering was placed above the slides (plate VI, figure 4).

In July and August, observations demonstrated that most active discharge occurred immediately following a rain. A point of interest was that comparatively few spores were caught at the time of a rain, irrespective of its duration. This might be explained by the fact that during a rain period the ostioles are covered by water, and the asci instead of bursting and discharging the spores, pass through the ostioles intact, as was found in the laboratory experiments. The length of the discharge period following rains which did not thoroughly soak the diseased bark was found to vary directly with the duration of the rain and the atmospheric humidity subsequent to the rain. As an average, spores were discharged from 20 to 30 hours following a heavy rain.

Plate VI, figure 5 may be used to illustrate the results obtained in the spore discharge studies. Slides 1 to 8 show the spore discharge obtained from perithecial material on tree No. 1, while Nos. 9 to 16 represent the discharge for the corresponding periods on tree No. 2. Slides 1 and 9, exposed for an eight-hour rain, show no macroscopically visible spore deposits, although some spores are present on the slides. Numbers 2 to 6, and 10 to 14 demonstrate the very heavy discharge during the first hours after the rain, and the gradual diminution to the twenty-fifth hour, after which the discharge is very meagre. Nos. 8 and 16 illustrate that no discharge occurred later than 13 hours after the thundershower. The period of discharge in this instance was something under 13 as compared with more than 25 hours following a heavy rain.

The fruiting bodies discharging spores in 1936 were examined in 1937, and it was evident that individual perithecial stromata were capable of discharging spores in two consecutive summers.

Inoculation Experiments

Methods of Inoculation

During the summer of 1934, 70 inoculations were made on the trunks of healthy *P. tremuloides* and *P. grandidentata* in a 10- to 15-year-old stand located 20 miles east of Toronto. The following materials were used as inoculum:

(1) Suspension of ascospores in water.

(2) Diseased bark.

(3) Squares of agar containing actively growing mycelium of cultures derived from:
   (*a*) single ascospores
   (*b*) numerous ascospores germinating and growing together
   (*c*) numerous conidia germinating and growing together
   (*d*) diseased tissue.

The methods of inoculation may be tabulated as follows:

(1) Inoculum placed on unwounded bark.

(2) Inoculum placed in wounds (burned and unburned):
   (*a*) vertical slits to the cambium
   (*b*) horizontal slits to the cambium
   (*c*) periderm removed exposing the cortex.

Checks were established on each tree, always placed on the trunk above the inoculation. The inoculations and checks were protected for a period varying from two weeks to one month, by placing moist cotton around the incision, and covering the whole with wax paper (plate VIII, figures 1 and 2).

PLATE VIII *(See opposite page)*

Inoculation experiments of *H. pruinatum* on aspen poplar.

FIGURES 1 and 2. Photographs illustrating method of preparing wound inoculations.

Note the square of inoculum placed in the horizontal incision. The inoculum was protected by placing moist cotton above and below the incision, and wrapping the whole in an oiled-paper covering.

FIGURE 3. Two, 6-months-old cankers resulting from artificial inoculations. The central twig bears the check wound. X 0·7

The inoculation wound for the canker on the right consisted of cutting a vertical slit through the bark of the cambium, while on the left a patch of the periderm was removed exposing green cortical tissues as the infection court. Observe the irregular, lobed, canker margins, and absence of fungus fruiting bodies.

FIGURE 4. Healed-over inoculation on *P. tremuloides*. X 0·3

The inoculation was made in the spring of 1934. A canker developed during the summer, but since that time growth has been inhibited by the formation of host callous tissue.

FIGURE 5. Stages in the development of the canker shown on the left of Figure 3. X 0·7

The drawings from left to right were made at the following dates:
July 16. 1934 (date of inoculation)
August 14th
August 30th
October 2nd
December 2nd.

Observe that cankered bark is evident at the incision margin one month following the date of inoculation. Also the irregular canker margin, and patches of diseased bark in advance of the canker body.

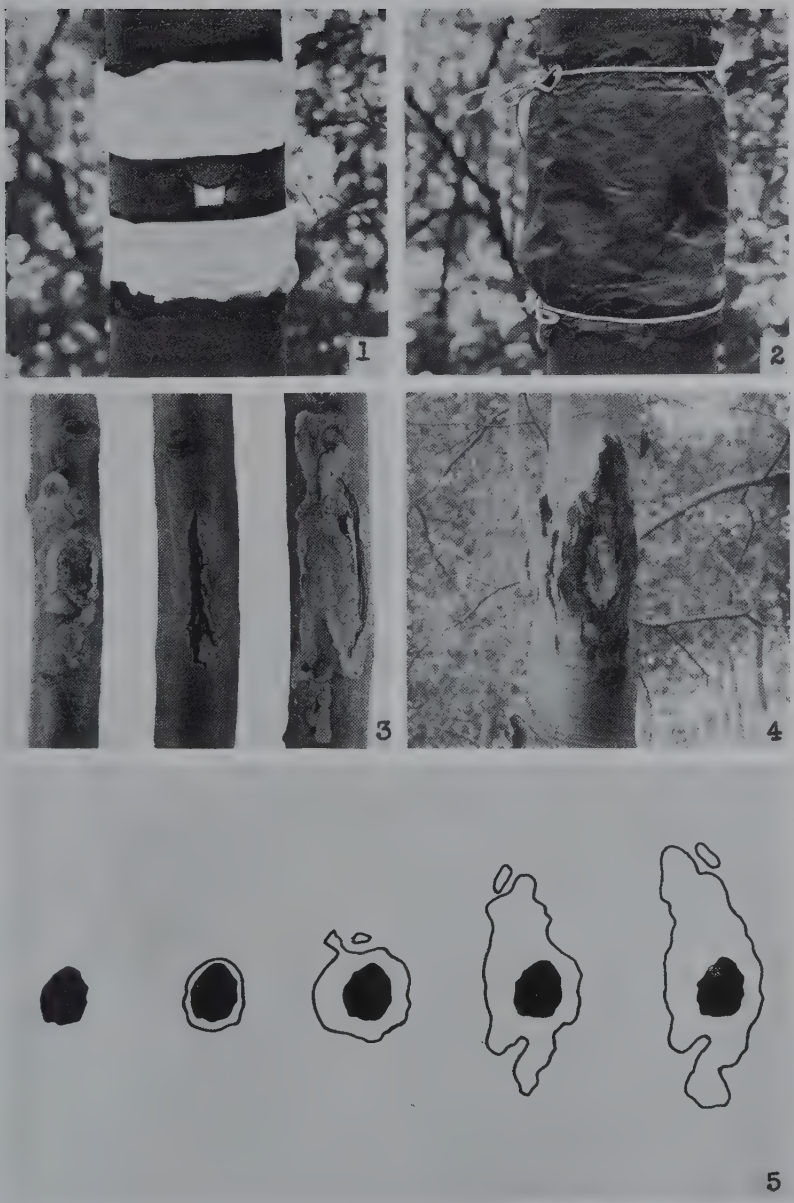


PLATE VIII (See opposite page)

Results of Inoculations

The inoculations on unwounded bark proved negative. The fungus grew over the periderm for some distance surrounding the inoculum, but was not able to penetrate and initiate an infection.

The wound checks remained sterile, and within a month the hosts had entirely closed the incisions with callous tissue.

The wound inoculations were uniformly positive when the inoculum consisted of mycelium of *H. pruinatum*. Negative results were obtained when ascospore suspensions were inserted into the wounds. These behaved similarly to the checks, that is, the wounds were healed by the formation of callous tissue a short time after the incisions were made. This wound tissue was not formed when mycelium was used as inoculum. Apparently the hosts were capable of forming callous tissue before the ascospores germinated and produced a body of mycelium sufficient to parasitize the living bark.

Killing the tissues by burning the incision margins was of no advantage in obtaining successful inoculations.

It was not necessary to have the inoculation wounds extend into the cambium as positive results were derived when only the superficial periderm was removed (plate VIII, figure 3).

P. tremuloides and *P. grandidentata* were proved susceptible to the disease.

Successful inoculations were made during the months of May, June, July, and August, 1934.

The following inoculation experiments were carried out at Ottawa during May and June of 1936.

Burned and unburned incisions on the trunks and branches of *P. tremuloides* were inoculated with:

(1) squares of potato dextrose agar containing numerous ascospores discharged from fresh perithecial material (excellent spore germination and growth was obtained in control plates).

(2) squares of potato dextrose agar containing actively growing mycelium from ascospore cultures.

Checks were established and a protection offered in the same manner as that employed in previous experiments.

Examination in November, 1936, demonstrated:

(1) Negative results when ascospores were used as the inoculum (incisions completely healed over).

(2) Positive results when mycelium was used as the inoculum (no callous tissue formed at the incisions).

(3) Checks remained sterile.

These results confirm the conclusions derived from the Toronto experiments.

Studies in the Development of Induced Cankers

The two cankers shown in plate VIII, figure 3, illustrate the character of incipient lesions resulting from inoculations. The inoculation incision on the right was cut through to the cambium (inoculation A), while on the left a small patch of the periderm was removed exposing green cortical tissue as the infection court (inoculation B). The central twig bears the check wound made on the same tree as the inoculations. A record of the rate of growth of these two inoculations is given in table 3. The outlines from left to right in plate VIII, figure 5, diagramatically represent the growth of canker B at the dates given in the table. It is evident that:

(1) Diseased bark is present at the incision margins one month following the date of inoculation.

TABLE 3.—GROWTH RECORD OF 2 INCIPIENT CANKERS RESULTING FROM ARTIFICIAL INOCULATIONS

Date of observation	Canker growth from the wound margins (inches)			
	Inoculation A		Inoculation B	
	Length	Width	Length	Width
July 16, 1934 (date of inoculation)	0	0	0	0
August 14, 1934	0·8	0·7	0·2	0·2
August 30, 1934	1·0	0·8	0·5	0·5
October 2, 1934	2·1	1·0	1·5	0·5
December 2, 1934	2·6	1·0	2·0	0·5

(2) Growth is more rapid along the trunk than across its circumference.

(3) Six months after inoculation, the cankers extended from two to three inches along the trunk from the wounds, and no fruiting bodies are evident on the diseased bark. These measurements are to be interpreted as average rates of growth. Some cankers grew faster, others slower than the above mentioned two. For example, the inoculation illustrated in plate IX, figure 1, produced a canker seven inches long after six months' growth.

(4) The advancing margins of the cankers are irregularly lobed, and patches of diseased bark occur in advance of the canker body.

The canker margin and yellowish to brownish diseased bark is very similar to that which occurs on natural cankers (plate III).

Three trunk cankers designated as C, D, and E will be described as representative of the more advanced cankers resulting from artificial inoculations. The methods and materials employed in making the inoculations and their subsequent rates of growth are given in table 4.

Considering first canker C, at the end of the first growing season the canker is seven inches long (plate IX, figure 1), and at the end of the second growing season, or 18 months after inoculation, the canker is 22 inches long and has spread approximately half-way around the trunk (plate IX, figure 2). Note the cracking of the bark at the margin of the first year's growth due to the formation of a callous layer. The diseased bark at this time is infested with the larvae of secondary bark beetles, and the black emergence holes are visible on the canker surface. Conidial fructifications are common but there is no evidence of the perfect stage. By the end of the third growing season or 29 months after inoculation, the canker is 38 inches long, and has encircled 80 per cent of the trunk's circumference (plate IX, figure 3). The spotted grey and black diseased bark is similar to that found on natural cankers (plate I). Observe the patches below the inoculation incision, where at some previous date, the periderm was removed exposing the conidial layer. The first immature perithecial stromata are now present on the canker. On May 23, 1937, or 36 months after inoculation, the individual perithecia were apparent, and ascospore discharge was taking place (plate IX, figure 4). The tree was girdled and killed by the fungus during the summer of 1937, that is, approximately three and one-half years after inoculation.

PLATE IX *(See opposite page)*

Inoculation experiments of *H. pruinatum* on aspen poplar.

FIGURES 1 to 4. Stages in the development of a canker resulting from artificial inoculation (canker designated as "C" in text).

FIGURE 1. Canker present 6 months after inoculation.

Note the vertical inoculation incision, and the irregular advancing canker margin. X 0.2

FIGURE 2. Canker present 18 months after inoculation.

The canker is now 22 inches long and has spread half way around the trunk. Note the cracking of the bark at the margin of the first year's growth, due to the formation of a callous layer. The diseased bark is infested with the larvae of secondary bark-boring beetles and the black emergence holes are evident on the cankered surface. X 0.2

FIGURE 3. Canker present 29 months after inoculation.

The canker is 38 inches long and has encircled 80 per cent of the trunk's circumference. Observe the spotted character of the diseased bark, and the patches below the inoculation incision where at some previous date the periderm was removed exposing the conidial layer. X 0.2

FIGURE 4. First perithecial stromata appeared on the canker 36 months after inoculation. XI

FIGURES 5 to 7. Stages in the development of a canker resulting from artificial inoculation (canker designated as "D" in text).

FIGURE 5. Canker present 15 months after inoculation. X 0·3

Observe the horizontal inoculation incision. Serves as an example of a late summer inoculation as compared to the spring inoculation described previously.

FIGURE 6. Canker present 26 months after inoculation. X 0·3

Note the rapid longitudinal growth without any great tangential spread.

FIGURE 7. Top half of canker shown in Figure 6, 38 months after inoculation. X 0·5

The first immature perithecial stroma is present (indicated by arrow), and observe its location with respect to the open "conidial blisters" near the upper margin of the canker.

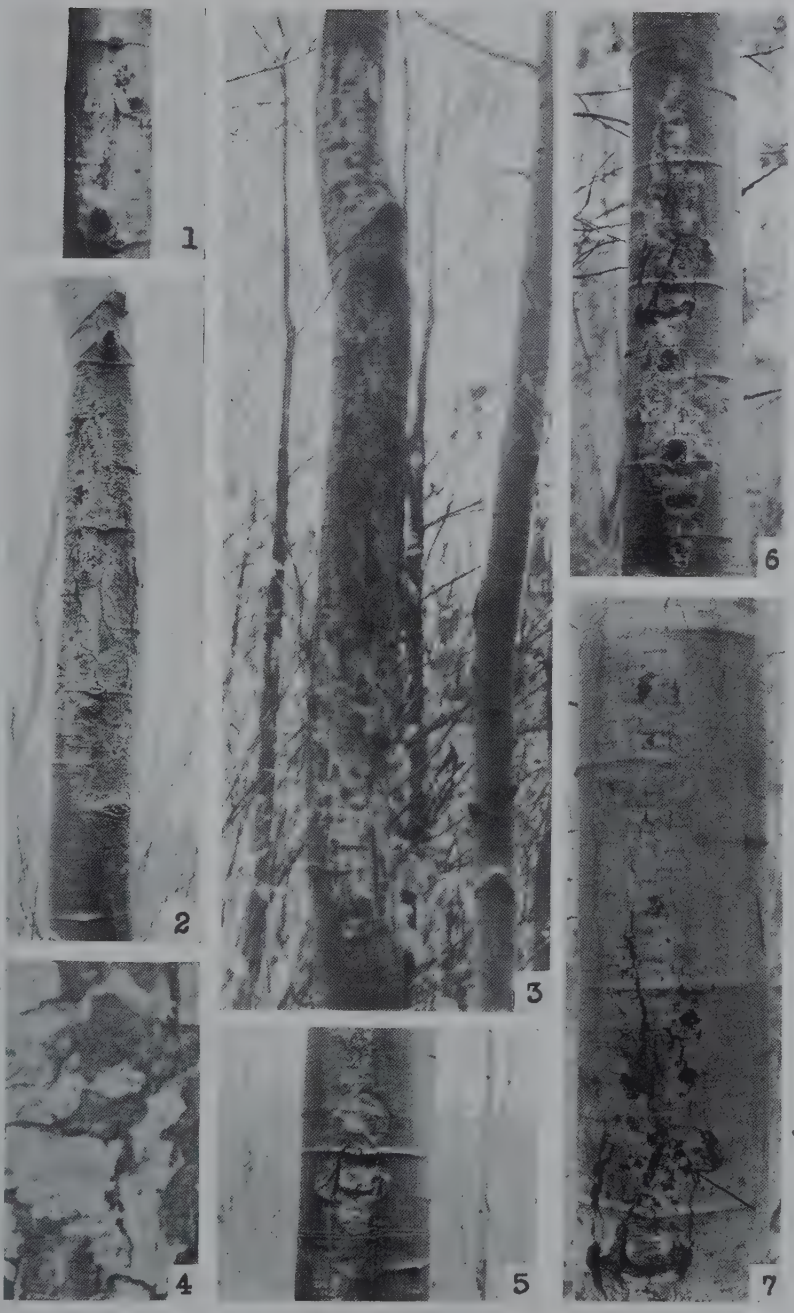


PLATE IX (See opposite page)

TABLE 4.—RECORD OF THE GROWTH OF THREE PERENNIAL CANKERS RESULTING FROM ARTIFICIAL INOCULATIONS

Canker	Host species	Type of incision	Inoculum	Date of inoculation	Subsequent canker growth					
					Nov. 25/34		Nov. 15/35		Oct. 17/36	
					Length of canker (inches)	% of circumference diseased	Length of canker (inches)	% of circumference diseased	Length of canker (inches)	% of circumference diseased
C.....	*P. trem.*	vertical slit	square of agar containing mycelium from multiple ascospore culture.	May 26/34	7	20	22	50	38	80
D.....	*P. grand.*	horizontal slit not burned	square of agar containing mycelium from tissue culture.	Aug. 14/34	no measurements		7	10	14	20
E.....	*P. grand.*	horizontal slit burned	square of agar containing mycelium from tissue culture.	Aug. 14/34	no measurements		5	10	18	10

Cankers D and E demonstrate cankers resulting from inoculations made in late summer as compared to the spring inoculation described previously. The lesions also illustrate positive results whether or not the tissues surrounding the inoculation incision are burned. Canker D shows to advantage how in some instances the cankers grow very rapidly along the trunk without extending appreciably around its circumference (plate IX, figure 6). At the top, note the patches of diseased bark isolated from the canker body. By October, 1937, or 38 months after inoculation, the first immature perithecial stroma was present on the canker (plate IX, figure 7). This structure was not evident in May, 1937. At this time the canker demonstrates the "conidial blisters" near the advancing margin and their position with respect to the first perithecial stroma.

Occasionally the host is able to surround entirely the cankered region with a callous layer, and in this way ward off further attack. Plate VIII, figure 4, shows an inoculation which produced a canker during the summer of 1934, but since that time growth has been stopped by the wound tissue.

Numerous cultures of *H. pruinatum* have been derived from diseased bark isolations made at the margins of artificially induced cankers.

It is important to state that inoculations to date, whether made during the spring, summer, or fall, produce their first perithecia approximately three years after inoculation. Further, in some cases the immature perithecial stromata are visible in the fall of the year, maturing the next spring, while in others the first sign of the perfect stage appears in the spring and matures during the summer. Present evidence indicates that the production of the perfect stage is not confined to any one season.

The Problem of Thinning in Aspen Stands

Aspen stands of from 15 to 25 years of age at the Petawawa Forest Experiment Station, which have been thinned during the last ten years, are at present affording some interesting considerations from a canker standpoint. All trunk-cankered trees were removed with other inferior material at the time of thinning. Since it was not practical to utilize the thinnings, the cut trees were piled and left on the area.

At the present time trees with Hypoxylon cankers are commonly found on the thinned areas. For the most part the cankers are located from 10 to 20 feet above the ground level. To protect the residual stand it is necessary to remove these cankered trees, an operation which will make openings in the crown cover and disturb the optimum stand density.

Applying present knowledge of Hypoxylon canker, the following suggestions are presented for consideration in the future thinning of aspen stands. In the first instance, trunk cankers frequently occur at such a height above the ground that they are very difficult to see when the leaves are on the trees. This would indicate that aspen areas to be thinned, should be examined after the leaves have fallen, in order that all possible cankered trees could be marked for removal. Further, since it is very hard to distinguish canker in its young stages, some diseased trees will probably remain after the first thinning. To allow for these and any new infections which may occur, the first thinning should be sufficiently moderate that the areas could again be thinned, say five years later, and the stand maintained at an optimum density. Another important point is that the *Hypoxylon* fungus may live as a saprophyte on the cankered trees that are cut and left on the area. Therefore, an enormous supply of inoculum capable of spreading the disease arises from this source, and in order to protect the stand this material should be destroyed.

Summary and Conclusions

Although the fungus *H. pruinatum* was originally described from a North American collection made prior to 1833, the first record associating the fungus with a canker disease of poplar was not made until 1920. During the last 18 years several investigators have reported the common occurrence of the disease in widely separated regions of Canada and the United States, from the Atlantic coast as far west as the province of Alberta, and in the state of Minnesota. The disease has been found in every stand examined in Ontario. These observations indicate that Hypoxylon canker has been present unobserved for many years and in the future will be found throughout the range of aspen poplar.

The young, smooth, green bark and cambium of aspen trunks and branches are invaded by the fungus. Cankers are formed which ultimately girdle and kill the diseased structures and cause the death of all distal parts. Cankers have been found on trees of all ages up to 65 years. In older trees the trunk cankers are located in the upper part of the bole, demonstrating that susceptibility is apparently not dependent on the age of the trees but on the age of the bark. The fungus has not been found attacking the thick, corky bark in the basal region of older trees. The cankers occur commonly on the healthy, fast-growing members of a stand, and are not in any way confined to suppressed or otherwise weakened trees.

In Ontario the disease is prevalent on *P. tremuloides* and *P. grandidentata*. Experiments are under way for the establishment of the pathogenicity on poplar of forms of *Hypoxylon* similar to *H. pruinatum* occurring on *Alnus, Betula, Salix, Acer,* and *Quercus*. Under field conditions these trees are often found in association with aspen.

Incipient cankers are invariably associated with some type of wound. In the Toronto region, insect punctures commonly serve as avenues of entrance. Canker growth is more rapid along trunks and branches than across their circumference. Trunk cankers on 10- to 15-year-old trees are usually three or more feet long before the trees are girdled and killed. The infected bark of older cankers is heavily infested with the larvae of secondary bark-boring beetles.

The fungus associated with the cankers is *Hypoxylon pruinatum* (Klot.) Cke.

The causal organism has been isolated into pure culture from ascospores, conidia, and diseased bark. Inoculations were made on wounded and unwounded bark of healthy aspen trees, employing mycelium from the above sources as the inoculum. The inoculations on unwounded tissue and the checks remained sterile. The inoculations on wounded tissue developed into cankers of the same character as those found in nature. Cultures of *H. pruinatum* have been obtained from diseased bark isolations made at the margins of artificially produced cankers. The imperfect fructifications become evident on the induced cankers at the end of the first or during the second growing season. The primary perithecial stromata were produced approximately three years after inoculation. Present evidence demonstrates that the perfect stage may form in the spring, summer, or fall and consequently is not confined to any one season. Trees ranging from four to seven inches in diameter were girdled and killed by the fungus three and one-half years after inoculation.

Natural cankers often produce the conidial stage during their first year's growth, but the perfect stage has been found only on cankers three or more years of age.

Spore germination and cultural studies were made from conidia and ascospores, and differences noted in the appearance of single ascospore cultures. Descriptions are presented of the colonies derived from the eight spores of a single ascus.

Studies were made on ascospore discharge in the field and under laboratory conditions.

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